Modelling fungal growth in heterogeneous soil: Analyses of the effect of soil physical structure on water distribution and fungal colonisation

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Abstract

Fungi play a pivital role in soil ecosystems contributing to plant productivity. The underlying soil physical and biological processes responsible for fungal colonistaion are interrelated and, at present, poorly understood. If these complex processes can be understood then this knowledge can be managed with an aim to providing more sustainable agriculture. Our understanding of microbial dynamics in soil has long been hampered by a lack of a theoretical framework and difficulties in observation and quantification. We will demonstrate how the spatial and temporal dynamics of fungi in soil can be understood by linking mathematical modelling with novel techniques that visualise the complex structure of the soil. The combination of these techniques and mathematical models opens up new possibilities to understand how the physical structure of soil affects water distribution which subsequently impacts on fungal colony dynamics. We will quantify, using X ray tomography, soil structure for a range of artificially prepared microcosms. We characterise the soil structures using soil metrics such as porosity, pore size distribution, and the connectivity of the pore volume. We use Lattice Boltzmann methods to predict the distribution of water in these soil microcosms. Furthermore we will use the individual based fungal colony growth model of Falconer et al. 2005, which is based on the physiological processes of fungi, to assess the effect of soil structure on water dynamics and microbial dynamics by qualifying biomass distributions. We demonstrate how soil structure can critically affect fungal colony growth and species interactions and how the distribution of water also effects this with consequences for biological control and fungal biodiversity.

Introduction

Fungi are a central component of the biosphere, essential for the growth of over 90 % of all vascular plants (Allen 1993), play an essential role in ecosystem services (Boumans 2002) and it is estimated that there may be as many as 1.5 million species of fungi globally (Hawksworth 1991). Fungi possess a unique biology driven by their inderminancy and plasticity resulting in a Fungal Ecology that is dependent on the macroscopic and microscopic worlds. This is especially true for soil systems where there is gap between the spatial scales at which soil ecosystem functions are observed and the scale at which microorganisms that underpin the majority of these processes operate. Development of a fungal ecology requires an understanding of the spatio-temporal growth and interaction dynamics of fungal communities at various scales. In particular, an understanding of the driving forces shaping ecologically important fungal communities in soils and their response to management practises is lacking. The aim of this work is to exploit recent advances in

modern techniques that characterise the soil environment at microscopic scales, and combine this with physiologically based models of fungal activity and theoretical techniques for predicting water distributions to develop a mechanistic framework for fungal growth dynamics in water filled heterogeneous soil environments.

The defining feature of fungi is their indeterminate life form and apical growth that provides opportunities to locate new carbon sources which can be translocated through the mycelial network to other parts of the fungal colony (Spiers at al 2009). Fungi (3–10 mm) are much larger in size relative to bacteria (0.5–1 mm) and as such fungi are rarely found in micropores (Killham, 1994). Given that there is a niche separation, with respect to pore sizes occupied for fungi and bacteria, this may allow them to coexist. Fungi must cope with additional factors that go with occupying macro-pores and these include being more vulnerable to predation and wet-dry cycles (Denef et al., 2001). Previous research examing how fungi explore the soil matrix has shown that 80–90% of fungi may be restricted to larger pores (Hattori (1988). This is consistent with later work by Harris et al. (2003) and Otten et al. (1999) that show preferential fungal exploration through larger and air-filled pores. The geometry of the pore-solid soil matrix not only effects fungal growth and colonisation but also governs fungal interactions. Soil structure is crucial in the interaction, we have previously shown that on a 2D agar plate (or any structure-less environment) two fungal species will always meet and they will compete. However in soil the 3D geometry of the pore space may provide refuges and separates pathways for fungal spread enabling species to coexist (Falconer et al 2007). It is not only the soil structure that effects fungal colonisation, fungi, rely on the coexistence of water and connected air filled pore spaces to permit their development and connectivity to the wider soil ecosystem (Spiers et al 209). Thus, the ability of soil to allow water to penetrate into it, and hold water is a key characteristic of all soil ecosystems as it effects microbial populations. The physical process of water distribution in soil can be determined by experiments, determining the well known moisture release curve, and using theoretical tools for modelling the reactive flow of multiphase compounds in soil at the pore scale such as the Lattice Boltzmann(LB) formulism. The moisture release curve defines the hydraulic and gaseous connectivity of soil ecosystems which is determined by the intricate geometry of the pore network. The advantage of the LB method is that it can take into account the 3D nature of the pore space and the resulting surface tension and contact angles of the solute and the capillary forces and potentially the hydrophilic nature of the soil.

It has been acknowledged that in order to understand the role of microbes in soil processes and to determine how the soil physical conditions affect fungal community dynamics new interdisciplinary and integrated approaches are required. (Spiers et al 2009). Towards this we outline a theoretical framework that integrates a set of physical and microbiological processes of soil. Below we outline how the integrated techniques for:
1) characterization of the micoscale soil environment
2) predicting the spatial distribution of water in soil
3) assessing the impact of soil structure and water distribution on fungal colonisation.

This integrated framework is a step towards developing an ecological theory for soil fungi where the micro-environment dynamics are modelled as an emergent consequence of the interactions between pore structure, physical processes (Carbon and water dynamics) and fungal growth and interactions. The physical and biological compartments are integrated in a 3D pore scale representation of soil obtained using X-ray CT.

Methods

Characterisation of soil structure using Computed Tomography

Soil samples were taken from experimental plots established at the Scottish Crop research Institute, Invergowrie, Dundee, on a Dystic-Fluvic Cambisol (FAO) with a sandy loam texture. From 2003 onwards, tillage operations were applied annually. Two soil samples were taken from the top 0-5 cm from fields which had received deep ploughing to a depth of 40cm and disking (P (P2 & P1)), and one sample was obtained from the field with zero tillage treatment where seeds have been drilled directly (N3). These samples were selected as these gave different pore-size distributions. Sample rings were scanned in a Metris X-Tek X-ray micro-tomography systems at 150 kV and 50 µA, a 2mm Al filter, and obtaining 1200 angular projections. The radiographs were reconstructed into a 3-D volume using CT-Pro (Nikon) at a resolution of 35 µm, imported into VGStudiomax and converted into image stacks with voxel thickness slices. Image stacks were imported into ImageJ. A median filter was applied prior to automated thresholding using the ISO-Data procedure in ImageJ. Small cubes sized 128 * 128 *128 were selected from the thresholded volumes to provide appropriate data sets for simulation which differed in pore size distributions.

Physical Properties

Physical properties of the prepared samples have been calculated using an ImageJ plug-in – SCAMP software for. Sample characteristics are:

I. porosity measures – the total number of voxels defined as pores divided by total volume of sample,
II. pore space connectivity – calculated using burning algorithm, assigning individual identifiers to each pore and checking the connections between them – it results in percentage of total pore space associated with the largest pore,
III. pore size distribution – calculated using the burning algorithm. A growing sphere is simulated at every voxel of pore space. The sphere increases its radius until it reaches a voxel corresponding to the solid phase. The radius of the sphere is recorded when all edges touch the solid material. It results in a percentage of each pore-size for each pore size class.

LB Model

The structure of the 3 soil samples (P1, P2 and N3) have been determined and are now used to predict the water distributions using the Lattice Boltzmann method, this is subsequently used as input into the fungal growth model, to determine the effect of structure on water distribution and subsequent fungal dynamics. The Shan Chen Single Component Multiphase (SCMP) Lattice Boltzmann model was implemented using open source software PALABOS (Sukop & Thorne (2006), Sukop & Or (2003)). The parameters used in the simulation were the same as cited Sukop & Or (2003) and also used in Basit & Basit (2010). For the three structures the SCMP model was run to determine the water distribution in pore space at equilibrium providing a water/air distribution in the soil microcosm based on the intricate geometry of the pore space.

Fungal Growth Model

In Falconer *et al*. (2005, 2007, 2008) we demonstrated the use of a physiologically-based model to explore the factors that influence the nature of fungal community diversity and the link between individual behaviour and the structure and function of fungal communities. The model is individual-based and incorporates the essential physiological processes of nutrient absorption, within colony biomass transport and recycling, inhibitor production and growth, and these occur differentially within a single mycelium as a consequence of local and non-local context. This differential behaviour permits different parts of the mycelium to expand and senesce concurrently. This framework was developed to capture the minimal set of physiological processes required to reproduce the observed range in phenotypic response in real colonies: uptake, redistribution of biomass, remobilisation of biomass, and growth which are known to be important for vegetative growth of fungi but have not collectively been incorporated into previous modelling frameworks (Falconer *et al*. 2005). We have also investigated the consequences of environmental heterogeneity for biomass distribution (Falconer *et al*. 2007), identifying which trait sets allowed individuals to persist in given environmental contexts. The model has been used to explore the effect of different soil management strategies on fungal invasion and interactions (Fig. 1; Kravchenko *et a*l. 2010). The enhancement of this model to incorporate inhibitor production that impacts inter-colony interactions is described in Falconer *et al*. (2008). The model was used to generate mycelial distribution maps that emerge from fungal interactions among a community of intrinsically different individuals (Falconer *et al.* 2010). This is the first attempt to model (physiologically) the dynamics of a fungal community in terms of a fungal ecology. We introduced the concept of a biomass-based abundance distribution function, described the form of that curve, and made the first attempt to identify the traits that affect the form of that curve. Ongoing developments are to apply the model to wet soil systems to understand the effect of physical and chemical processes on fungal diversity. We use this model to explore the effect of fungal colonisation on the samples described in 2.1) above with water/air distribution as derived from 2.2) and also a completely dry soil sample i.e. only air filled pores and no water present. The only change to the fungal model is how the fungi respond to the presence of water. Consistent with Otten et al 1999 we reduce the diffusion coefficient, governing colony spread, where there is a high density of water and map the diffusion coefficients linearly onto the fluid density. The spread of fungi is now a function of water distribution and structure.

Results

Physical Properties

Sample	Bulk porosity	Connected fraction
N3	0.05887	62.84
P1	0.27430	97.25
P2	0.34456	98.78

The table shows that the three samples vary in their porosity, P1 and P2 are relatively porous and N3 has lowest poristy. The pore space for all samples is well connected.

Effect of LB on D coefficients

For the P2 sample we map the diffusion coefficients for fungal spread in a pore network which is completely air filled , here there are only two values for diffusion coefficient (0 & 255), this maps to a solid and air-filled voxel and is shown in Fig 1a. In Fig 1b we show the diffusion coefficients mapped to density of fluid (water). The diffusion coefficients range from 0 to 255. We can see that large sections of the pore space is water filled (dark blue pixels) as predicted by SCMP Lattice Boltzmann method, and these areas are less likey to be invaded by fungal colony. This fundamentally alters the connectivity of the pore volume and may have consequence for fungal colonisation and interactions.

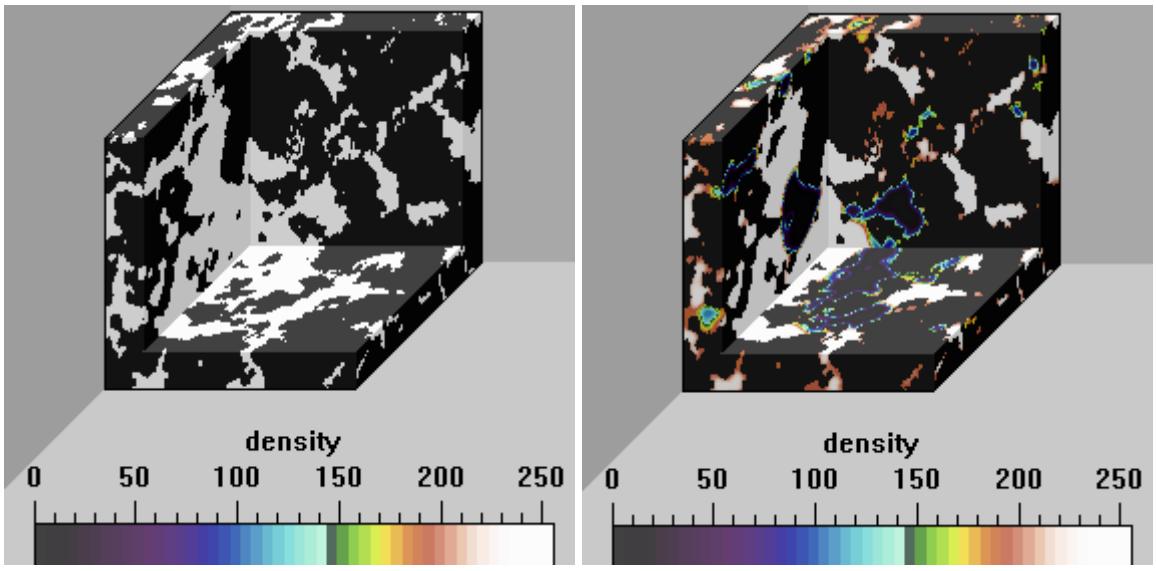

Figure 1Distribution of D values for (a) soil sample with no water (b) with water. Black and white voxel corresponds solid and pore voxel respectively. b) shows the additional diffusion coefficient distibution as linearly mapped to water distribution as predict

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Effect of water filled and dry soil on fungal colonisation

Fungal invasion was initiated from the first z-y plane of the 3D sample. The fraction of pore volume occupied by fungal biomass was calculated per segment. The volume was split into 9 equal segments and for each segment the porosity and fraction of pore space occupied by biomass was determined. This allows quantification of fungal invasion over space and time. See Fig 2 for an example of how the segments relate to the volume. This figure is reproduced from Pajor et al 2010. Plots of fungal invasion for samples P2 and N3 are shown in Fig 3 and 4. Fig 5 combines all of the plots in Fig 3 and Fig 4 to illustrate how both the structure and water distribution effect colony growth.

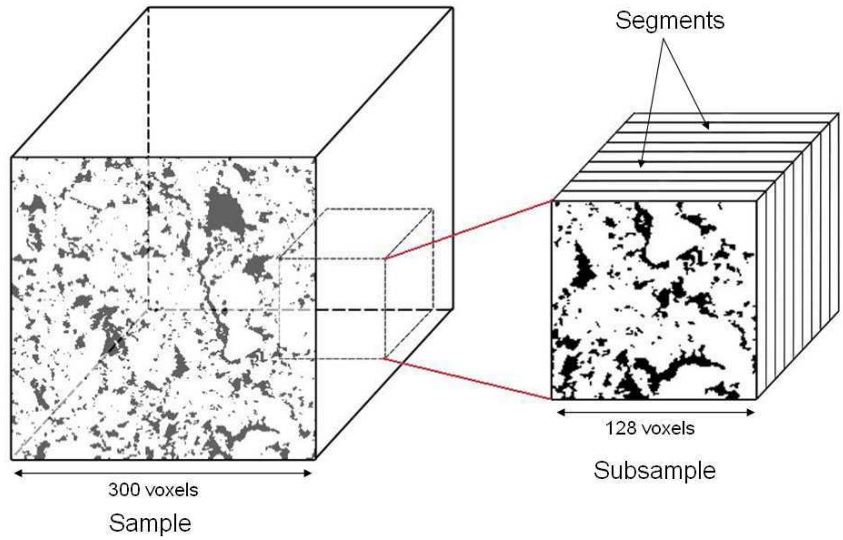
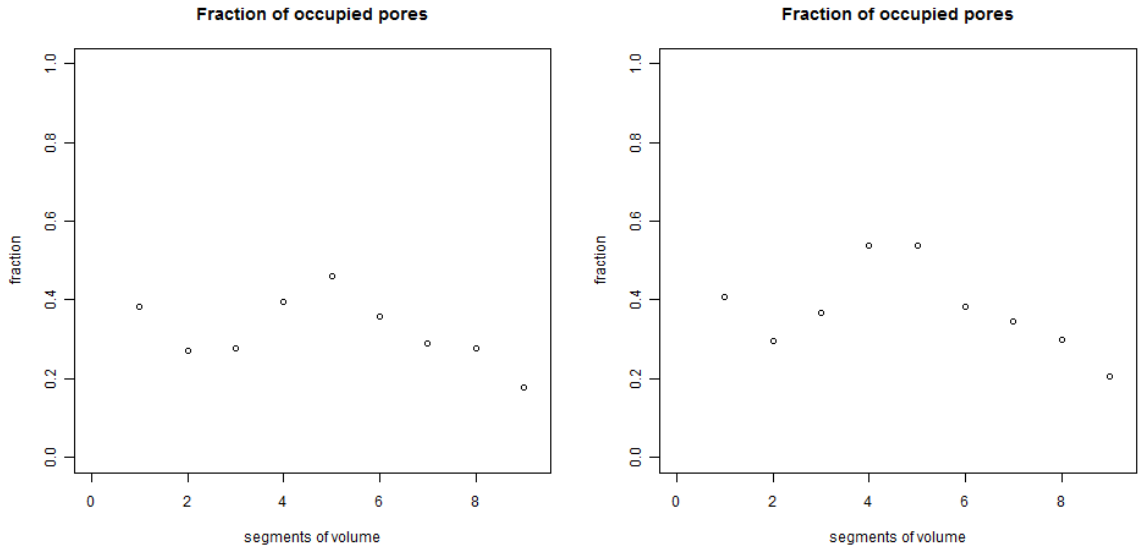


Figure 2 3-D spatial arrangement of the data structure. Treatments, P2, P1 and N3 were compared by comparing physical properties for cubed samples. The sample was divided into segments to enable quantification of fungal invasion over space and time.



(a) (b)

Figure 3 The fraction of biomass occupied pores for sample P2 a) with water b) dry sample

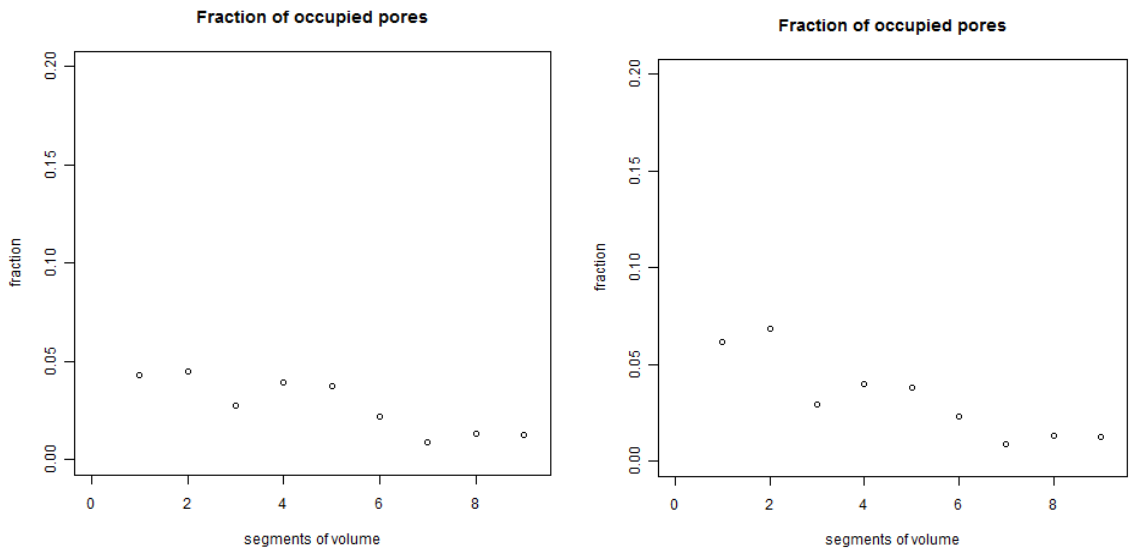


Figure 4 The fraction of biomass occupied pores for sample N3 a) with water b) dry sample

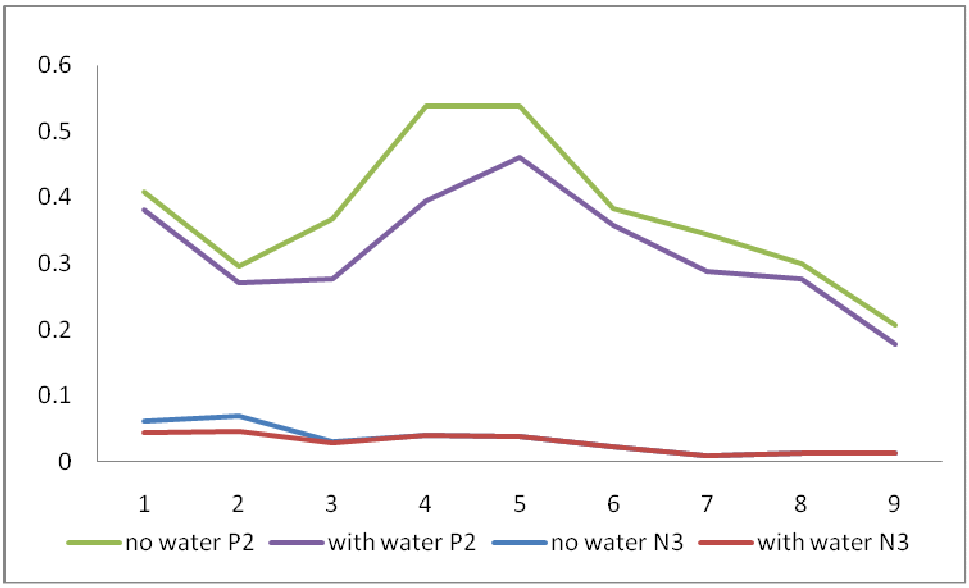


Figure 5 changes in the fraction of pore space occupied by biomass (y axis) in each segment with increasing distance from the site of inoculation, for soils P2 and N3 with and without presence of water.

As can be seen from the graphs in Fig 5 we can see both soil structure and presence of water effect the colonisation dynamic of fungi. It seems however that some structures (N3) is less sensitive to the presence of water that (P2) and this can possibly be explained by the structural characteristics of the soil.

Conclusion

As can be seen from the graphs the two samples for treatment N3 and P2 are different in their porosity and connectivities. This has implications for how water will be distributed throughout the pore space. From Fig 1 we can see how the presence of water will affect the diffusion coefficients that drive fungal spread for sample P2. Voxels with a high water density are mapped to a low spread diffusion coefficient and can be considered impenetrable

by the fungi. This has the consequence of altering the connectivity of the pore geometry which will effect fungal invasion. Fig 5 shows that water and structure effect fungal colonisation of the pore space.

References

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